PATENT COOPERATION TREATY

PCT

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Article 36 and Rule 70)

REC'D 28 MAY 2004
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Applicant's or agent's file reference MICC/P28321PC	**FOR FURTHER ACTION**	See Notification of Transmittal of International Preliminary Examination Report (Form PCT/IPEA/416)
International application No. PCT/GB 03/01756	International filing date *(day/month/year)* 24.04.2003	Priority date *(day/month/year)* 24.04.2002

International Patent Classification (IPC) or both national classification and IPC
G01N21/64

Applicant
IMPERIAL COLLEGE INNOVATIONS LIMITED et al.

1. This international preliminary examination report has been prepared by this International Preliminary Examining Authority and is transmitted to the applicant according to Article 36.

2. This REPORT consists of a total of 10 sheets, including this cover sheet.

   ☒ This report is also accompanied by ANNEXES, i.e. sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications made before this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions under the PCT).

   These annexes consist of a total of 5 sheets.

3. This report contains indications relating to the following items:

   - I ☒ Basis of the opinion
   - II ☐ Priority
   - III ☒ Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
   - IV ☐ Lack of unity of invention
   - V ☒ Reasoned statement under Rule 66.2(a)(ii) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
   - VI ☐ Certain documents cited
   - VII ☐ Certain defects in the international application
   - VIII ☐ Certain observations on the international application

Date of submission of the demand	Date of completion of this report
19.11.2003	27.05.2004

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INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No. PCT/GB 03/01756

I. Basis of the report

1. With regard to the **elements** of the international application *(Replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to this report since they do not contain amendments (Rules 70.16 and 70.17))*:

Description, Pages

1-3, 5-14	as originally filed
4, 4a	filed with telefax on 20.04.2004

Claims, Numbers

1-12	filed with telefax on 20.04.2004

Drawings, Sheets

1/3-3/3	as originally filed

2. With regard to the **language**, all the elements marked above were available or furnished to this Authority in the language in which the international application was filed, unless otherwise indicated under this item.

   These elements were available or furnished to this Authority in the following language: , which is:

   ☐ the language of a translation furnished for the purposes of the international search (under Rule 23.1(b)).

   ☐ the language of publication of the international application (under Rule 48.3(b)).

   ☐ the language of a translation furnished for the purposes of international preliminary examination (under Rule 55.2 and/or 55.3).

3. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international preliminary examination was carried out on the basis of the sequence listing:

   ☐ contained in the international application in written form.

   ☐ filed together with the international application in computer readable form.

   ☐ furnished subsequently to this Authority in written form.

   ☐ furnished subsequently to this Authority in computer readable form.

   ☐ The statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.

   ☐ The statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished.

4. The amendments have resulted in the cancellation of:

   ☐ the description, pages:

   ☐ the claims, Nos.:

   ☐ the drawings, sheets:

5. ☐ This report has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed (Rule 70.2(c)).

   (Any replacement sheet containing such amendments must be referred to under item 1 and annexed to this report.)

6. Additional observations, if necessary:

III. Non-establishment of opinion with regard to novelty, inventive step and industrial applicability

1. The questions whether the claimed invention appears to be novel, to involve an inventive step (to be non-obvious), or to be industrially applicable have not been examined in respect of:

   ☐ the entire international application,

   ☒ claims Nos. 9,12

   because:

   ☐ the said international application, or the said claims Nos. relate to the following subject matter which does not require an international preliminary examination (specify):

   ☒ the description, claims or drawings *(indicate particular elements below)* or said claims Nos. 9,12 are so unclear that no meaningful opinion could be formed *(specify)*:

   see separate sheet

   ☐ the claims, or said claims Nos. are so inadequately supported by the description that no meaningful opinion could be formed.

   ☐ no international search report has been established for the said claims Nos.

2. A meaningful international preliminary examination cannot be carried out due to the failure of the nucleotide and/or amino acid sequence listing to comply with the standard provided for in Annex C of the Administrative Instructions:

   ☐ the written form has not been furnished or does not comply with the Standard.

   ☐ the computer readable form has not been furnished or does not comply with the Standard.

V. Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Yes:	Claims	8
	No:	Claims	1-7,10,11
Inventive step (IS)	Yes:	Claims	
	No:	Claims	8
Industrial applicability (IA)	Yes:	Claims	1-12
	No:	Claims	

2. Citations and explanations

see separate sheet

Re Item III

Non-establishment of opinion with regard to novelty, inventive step and industrial applicability

1. **Claims 9 and 12** rely on references such as: "as described in part... of the description" or "as illustrated in the drawings".
The claims must not, in respect of the technical features of the invention rely on references to the description or drawings "except where absolutely necessary". In such an exceptional case, the applicant is invited to show that it is "absolutely necessary" to rely on reference to the description or drawings (see also the PCT Guidelines, III-4.10).

Re Item V

Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

2. Reference is made to the following documents:

D1: US-A-6 008 892
D2: JP(A) 2000151916, PATENT ABSTRACT OF JAPAN
D3: DE-U-29700253
D4: US-B1-6 369 893
D5: JP(A) 11304707, PATENT ABSTRACT OF JAPAN

3. **Novelty (Art. 33(2) PCT) and Clarity (Art. 6 PCT):**

Apparatus claims 1-7 and **method claims 10 and 11** do not meet the criteria of Article 33(2) PCT with respect to novelty for the following reasons:

3.1 **Clarity** (Art. 6 PCT) and interpretation of **Claim 1**:

(a) The expression "**that illuminates**", used in lines 11 and 14 of **apparatus** claim 1, relates to a **method** of using the apparatus rather than clearly defining the apparatus in terms of its technical features. The intended

limitations are therefore not clear from this claim, contrary to the requirements of Article 6 PCT. It is presumed, that a LED "**for** illuminating" is meant (see PCT Guidelines III-4.8).

(b) Claim 1 defines "an illuminator *(suitable)* ***for*** (see PCT Guidelines III-4.8) illuminating a material". "**The material**" does, therefore, **not form part of the claimed invention**, since the claim does not define "A device... comprising... a material".

(c) The claim does not define any dimensions, for example, the beam spot size at the surface of the material, or the feature (microspot) size of the material. Since the material does also not form part of the claimed invention (see above), the **relative dimensions** and therefore the illumination/imaging **resolution** are not defined in claim 1.

(d) Claim 1 defines that the illuminator simultaneously illuminates all, **or (alternatively)** "a substantial portion".

(e) D1 discloses a scanning mechanism to scan the **whole** sample area (col. 4, lines 43-55). In col. 4, lines 45-46, it is stated that "**any** scanning mechanism that produces a two-dimensional scan may be used", and in col. 4, lines 56-57, it is mentioned that "the scanning beam is directed... to illuminate a spot, line or area" Therefore, in D1, at each scan position light is directed to a spot, line or area. Scanning is needed to illuminate neighboring or the next scanning positions.
In the present invention "a material comprising a **microarray assay** (whole sample area) **comprising a plurality of microspots** (scanning position spots)" is illuminated, whereby "the illuminator simultaneously illuminates all, or a substantial portion of **one** of the microspots". In order to illuminate a next individual microspot of the plurality of microspots of the microarray, and finally to illuminate the whole microarray, **the present invention also needs a scanning mechanism**.

(f) Although it is stated in D1, col. 4, lines 35-37, that "preferably" a coherent laser source is used, it is **explicitly mentioned** that "a non-coherent source, such as a light emitting diode (LED) could be used".

(g) The claim does not define to use "non-collimated" light, but non-coherent light.

(h) Although the **description** (page 3) of the present invention sets out disadvantages of the application of CCDs as detector devices, **claim 1** does not exclude, that the detector is a CCD array.

3.2 Document **D1** discloses (the references in brackets refer to D1):
- A device (fig. 1; col. 4, lines 24-34) [for reading fluorescent signals];
- an illuminator 18 (col. 4, line 34) [**for** illuminating a material 29 (fig. 1), 55 (fig. 2; col. 5, lines 35-38) bound with a fluorophore (fig. 2) at an appropriate wavelength to induce fluorescence (col. 5, lines 2-4)];
- a detector 39 (col. 5, lines 5-26) [for detecting fluorescent signals emitted by the material];
- a signal processor (implicitly disclosed: col. 1, lines 6-7, "examining, indicating, analysing, identifying". The signal detected by the PMT 39 has to be further processed) [for processing the signals detected];
- the device defining an optical system (see fig. 1) having an excitation optical path (col. 4, line 34 - col. 5, line 4) and a detection optical path (col. 5, line 5 - col. 5, line 26);
- the illuminator 18 comprises a light emitting diode (col. 4, lines 37-38; "a noncoherent source, such as a light emitting diode (LED) could be used") [*for* (see PCT Guidelines III-4.8) illumina*ting* the material with incoherent illumination];
- the illuminator 18 *is provided [for* (see PCT Guidelines III-4.8) illumina*ting* all, **or a substantial portion** of the material simultaneously (col. 4, line 57)], [*the material* (not part of the claimed invention) comprises a microarray assay comprising a plurality of microspots; the material is deposited on a substantially flat surface 53 (fig. 2; col. 5, lines 35-37)].

The subject-matter of **claim 1** is therefore not new and does not meet the criteria of Art. 33(2) PCT.

3.3 It should be noted, that **D2** also discloses all technical features of claim 1 (see figure 1 and abstract; LED 30, detector 51, processor (implicit) 52, 70, 80). The illuminator 30 simultaneously illuminates "all or a substantial portion" of the sample gel 11 (see figure).

Additionally **D3 and D4** disclose all technical features of claim 1 (see detailed relevant passages indicated in the search report). D3 discloses an apparatus suitable for reading the fluorescence of substances on liquid or solid surfaces (page 1, lines 1-5) illuminated with a LED (page 4, line 15). With respect to D4, which discloses a system for reading fluorescent signals from reaction vessels, reference is made to the comments in paragraphs 3.1 b)-d).

The subject-matter of **claim 1** is therefore also not new and does therefore not meet the criteria of Art. 33(2) PCT.

3.4 What has been said above with reference to apparatus claim 1 concerns **method claims 10 and 11** mutatis mutandis. It is in particularly referred to the comments provided in paragraphs 3.1 b)-d) above.

3.5 **D1** further discloses the subject-matter of the following dependent claims (the references in brackets refer to D1):
- **Claim 2:** excitation filter 20 (col. 4, lines 40-42) to filter out longer wavelengths emitted by the LED before they reach the material;
- **Claim 3:** short band pass interference filter 20 (col. 4, lines 40-42; the filter reduces "all unwanted wavelengths", this is achieved typically by using an Fabry Perot interference filter. For excitation of the fluorophores, light with high energy and short wavelength is needed);
- **Claim 4:** emission filter 23 or 34 (col. 5, lines 17-24) positioned in the detection optical path (see fig. 1) to filter out any directly reflected illumination ("reflecting the incident beam wavelength(s)");
- **Claim 5:** glass slide (col. 7, lines 38-41;
- **Claim 7:** polarising beam splitter 23 (col. 5, lines 21-22).

D2 further discloses the subject-matter of **dependent claim 6** (the references in brackets refer to D2):
- polarising filter 35 in the excitation path (see figures 1 and 2);
- second polarising filter 36 in the detection optical path (see figures 1 and 2) and oriented at right angles to the first polarising filter (see figure 2);

The subject-matter of **claims 2-7** does therefore also not meet the criteria of Art. 33(2) PCT.

4. Inventive Step (Art. 33(3) PCT):

Apparatus claim 8 does not meet the criteria of Article 33(3) PCT with respect to inventive step for the following reasons:

Document **D1**, which is considered to represent the most relevant state of the art, discloses, as indicated in paragraph 2.1, all technical features of claim 1. The subject-matter of claim 8 differs from D1 in that also a "**phase sensitive detector**" is disclosed.

This technical feature has the technical effect of increasing the signal-to-noise ratio in order to provide more accurate fluorescence measurement results. It is related to the **technical problem** of "phase difference drift" (see title of **D5**) between the phases of the fluorescence and exciting light. The skilled person using the system of D1 finds a **solution** for this problem in D5, in which a fluorescence measurement system is described, comprising a modulated light source and a phase detector 79.

Therefore, the skilled person would combine the phase detector arrangement of D5 with the system of D1 and would arrive at the subject-matter of claim 10 without involving an inventive step (Article 33(3) PCT).

It should be noted that the **"lock-in"** (see description, page 9, lines 19-20 of the present application) **technique is generally known** in the field of spectroscopy, in particularly for spectroscopical measurements of low intensity signals, where the skilled person has to face poor signal-to-noise ratios.

5. Industrial applicability (Article 33(4) PCT):

The requirement of Art. 33(4) PCT as to industrial applicability is fulfilled for **claims 1-8, 10, 11**.

6. Further comments (for the sake of completeness):

6.1 A lack of clarity can arise with **bracketed expressions in the claims** that do not include reference signs. In **claim 10** the bracketed expression **"(LED)"** is used.

6.2 Contrary to the requirements of Rule 5.1(a)(ii) PCT, **the relevant background art** disclosed in the **documents D1-D4** is not mentioned in the description, nor are these documents identified therein.

6.3 The features of the claims are not provided with **reference signs** placed in parentheses (Rule 6.2(b) PCT).

CLAIMS

1. A device for reading fluorescent signals comprising:

an illuminator for illuminating a material bound with a fluorophore, at an appropriate wavelength to induce fluorescence;

a detector for detecting fluorescent signals emitted by the material;

a signal processor for processing the signals detected;

the device defining an optical system having an excitation optical path and a detection optical path;

characterised in that the illuminator comprises a light emitting diode that illuminates the material with incoherent illumination;

the material comprises a microarray assay comprising a plurality of microspots; the material is deposited on a substantially flat surface and the illuminator simultaneously illuminates all, or a substantial portion of one of the microspots.

2. A device according to Claim 1 further comprising an excitation filter positioned in the excitation optical path to filter out longer wavelengths emitted by the LED before they reach the material to be analysed.

3. A device according to Claim 2 wherein the excitation filter comprises a short band pass interference filter.

4. A device according to any one of the preceding claims further comprising an emission filter positioned in the detection optical path to filter out any directly reflected illumination from the material.

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5. A device according to any one of the preceding claims wherein the substantially flat surface comprises a glass slide.

6. A device according to any one of the preceding claims further comprising a polarising filter positioned in the excitation optical path to be perpendicular to the input polarisation, and
a second polarising filter positioned in the detection optical path and orientated at right angles to the first polarising filter such that the two filters comprise crossed polarisers positioned in the excitation and the detection optical paths respectively.

7. A device according to any one of Claims 1 to 5 further comprising a polarising beam splitter positioned to lie in both the excitation and detection optical paths.

8. A device according to any of the preceding claims wherein the signal processor comprises a phase sensitive detector.

9. A device substantially as hereinbefore described with reference to the accompanying drawings.

10. A method of analysing signals emitted from a sample of material bound with a fluorophore, the method comprising the steps of:

illuminating the sample at an appropriate wavelength to cause fluorescence in the sample;

detecting fluorescent signals emitted by the sample once the sample has been illuminated;

analysing signals detected by the detector,

characterised in that the sample is illuminated with incoherent illumination using a light emitting diode (LED), the material comprises a microarray assay comprising a plurality of microspots; the material is deposited on a substantially flat surface and in that all, or a substantial portion of one of the microspots is illuminated simultaneously.

11. A method of analysing signals emitted from a sample of material bound with a fluorophore using a device according to any one of Claims 1 to 10.

12. A method substantially as hereinbefore described with reference to the accompanying drawings.

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According to a first aspect of the present invention there is provided a device for reading fluorescent signals comprising:

an illuminator for illuminating a material bound with a fluorophore, at an appropriate wavelength to induce fluorescence;

a detector for detecting fluorescent signals emitted by the material;

a signal processor for processing the signals detected;

the device defining an optical system having an excitation optical path and a detection optical path;

characterised in that the illuminator comprises a light emitting diode that illuminates the material with incoherent illumination;

the material comprises a microarray assay comprising a plurality of microspots; the material is deposited on a substantially flat surface and the illuminator simultaneously illuminates all, or a substantial portion of one of the microspots.

A method of analysing signals emitted from a sample of material bound with a fluorophore, the method comprising the steps of:

illuminating the sample at an appropriate wavelength to cause fluorescence in the sample;

detecting fluorescent signals emitted by the sample once the sample has been illuminated;

analysing signals detected by the detector,

characterised in that the sample is illuminated with incoherent illumination using a light emitting diode (LED), the material comprises a microarray assay comprising a plurality of microspots; the material is deposited on a substantially flat surface and in that all, or a substantial portion of one of the microspots is illuminated simultaneously.

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Existing systems for reading fluorescent signals particularly from microarray assays have all been imaging systems which produce high resolution image of the microarray, typically comprising over 400 pixels for subsequent analysis.

To achieve the signal to noise levels required to measure the signal from each pixel comprising the image, it had been thought necessary to use a coherent laser light source of relatively high power to illuminate the material but generally such lasers are expensive and excitation wavelengths

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